Antidepressant Effect of Fluoxetine Versus Imipramine in Diabetic Rats Exposed to Chronic Restraint Stress Hippocampal Monoamines Turnover and Inflammatory Markers

A proposal of a thesis submitted for partial fulfilment of Master Degree in pharmacology

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بسم الله الرحمن الرحيم

قَالُوا سُبْحَانَكَ لَا عِلْمَ لَنَا إِلَّا مَا عَلَّمْتَنَا إِنَّكَ

أَنتَ الْعَلِيمُ الْحَكِيمُ

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List of Abbreviations

5-HIAA	*5 hydroxyindolacetic acid*
5-HT	*Serotonin*
5-HT2	*5-hydroxytryptamine receptor2*
ACTH	*Adrenocorticotropic Hormone*
AGEs	*Advanced Glycation End products*
AMPA	*α-Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid*
ANOVA	*Analysis Of Variance*
AVP	*Arginine vasopressin*
BBB	*Blood Brain Barrier*
BDNF	*Brain Derived Neurotrophic Factor*
CNS	*Central Nervous System*
COX-2	*Cyclo-oxygenase-2*
CRF	*Corticotropin-releasing factor*
CRH	*Corticotropin releasing hormone*
CRS	*Chronic Restraint Stress*
CSF	*Cerebrospinal fluid*
DA	*Dopamine*
DAMPs	*Damage Associated Molecular Patterns*
db/db mice	*Leptin resistant mice obese*
DM	*Diabetes Mellitus*
EDTA	*Ethylenediaminetetraacetic acid*
ELISA	*Enzyme Linked Immunosorbent Assay*
FBG	*Fasting Blood Glucose*
FBI	*Fasting Blood Insulin*
FFA	*Free Fatty Acid*
FLU	*Fluoxetine*
FST	*Forced Swim Test*
GABA	*γ-Aminobutyric acid*
GH	*Growth hormone*
GR	*Glucocorticoid receptor*
HbA1C	*glycosylated haemoglobin*
HDL	*High Density Lipoproteins*
HFD	*High Fat Diet*
HOMA	*Homeostatic Model Assessment*
HPA	*hypothalamo-pituitary-adrenal*
HPLC	*High-performance liquid chromatography*
HVA	*Homovanillic acic*
i.p.	*Intraperitoneal*

IFN γ	*Interferon–gamma*
IGF-1	*Insulin-like growth factor 1*
IHD	*Ischemic Heart Disease*
IL-1	*Interleukin-1*
IL-10	*Interleukin-10*
IL-13	*Interleukin 13*
IL-1ß	*Interleukin-1beta*
IL-4	*Interleukin 4*
IL-6	*Interleukin-6*
IMIP	*Imipramine*
iNOS	*Inducible Nitric Oxide Synthase*
IR	*Insulin Resistance*
ITT	*Insulin Tolerance Test*
JNK	*c-Jun amino-terminal Kinase*
LDL	*Low Density Lipoproteins*
LPS	*Lipopolysaccharide*
MAO	*Monoamine oxidase*
MAOI	*Monoamine oxidase inhibitor*
MCP-1	*monocyte chemotactic protein 1*
MHPG	*3-Methoxy-4-hydroxyphenylglycol*
MI	*Myocardial Infarction*
MR	*Mineralocorticoid receptor*
mRNA	*Messenger RNA*
NA	*Noradrenaline*
NADPH	*Nicotinamide adenine dinucleotide phosphate*
NE	*Norepinephrine*
NF-ĸB	*Nuclear factor kappa B*
NMDA	*N-Methyl-D-aspartate*
NO	*Nitric Oxide*
NRC	*National Research Centre*
OFT	*Open Field Test*
OxLDL	*oxidized Low Density Lipoprotein*
PAD	*Peripheral arterial disease*
PAMPs	*Pathogen-Associated Molecular Patterns*
PGE2	*Prostaglandin E2*
RAGE	*Receptor for Advanced Glycation End products*
ROS	*Reactive Oxygen Species*
SBP	*Systolic Blood Pressure*
SD	*Standard Deviation*
SEM	*Standard Error of Mean*
SIT	*Social Interaction Test*

SSRIs	*Selective Serotonin Reuptake Inhibitors*
STZ	*Streptozotocin*
TC	*Total Cholesterol*
TCAs	*Tricyclic Antidepressants*
TGL	*Triglycerides*
TLR2	*Toll Like Receptor-2*
TLR4	*Toll Like Receptor-4*
TLR6	*Toll Like Receptor-6*
TLRs	*Toll Like Receptors*
TNF-α	*Tumour Necrosis Factor- alpha*
UCMS	*Unpredictable Chronic Mild Stress*
UK	*United Kingdom*
USA	*United States of America*
WHO	*World Health Organization*

INTRODUCTION

The concurrence of depression and diabetes is a serious problem. Among people with diabetes, whose risk of depression is 50–100% greater than the general population ***(Rubin et al., 2008)*** depression is associated with higher complication and mortality rates ***(Katon et al., 2005).*** Moreover, depression may impair control of glycemia and treatment compliance, as well as increasing the risk of vascular complications in diabetes ***(Gomez et al., 2001).***

Several attempts to investigate the neurobiology of depression and to measure antidepressant effects of drugs have made use of application of chronic stress, which is one of the major determinants in development of human depression. Humans with poorly controlled diabetes exhibit hyperactivation of the hypothalamic–pituitary–adrenal (HPA) axis, resulting in elevated circulating levels of cortisol ***(Desrocher M,Rovet J.,2004; Messier C., 2005).*** Similarly, levels of adrenal glucocorticoids are elevated in rodents with experimental diabetes ***(Magarinos and McEwen, 2000; Watts et al., 2005)***. The specific mechanism by which diabetes results in hyperactivation of the HPA axis is unknown, but is apparently not the result of the hyperglycemia per se ***(Chan O, et al., 2005)***. In addition, chronic stress and high levels of corticosterone can impair synaptic plasticity ***(Alfarez DN, et al., 2003)*** and inhibit neurogenesis in the hippocampus of adult rats ***(Gould E, et al. 1992)*** and lifelong levels of corticosterone are

correlated with age–related declines in neurogenesis and memory ***(Montaron MF, et al., 2006)***. It is therefore possible that elevated corticosterone levels in diabetes may mediate central impairment of neuronal structure and function. Hence, hyperglycemia may lead to imbalance of the hypothalamo-pituitary-adrenocortical axis and increase cortisol levels, as observed in depression.

In the brain, serotonergic fibres acts on specific receptors to modulate the activity on autonomic pathways and affects energy expenditure regulated by 5-HT receptors. Serotonergic pathways also directly affect glucose homeostasis through regulation of autonomic efferents and action on peripheral tissues ***(Daniel et al., 2007)***.The changes in brain 5-HT synthesis rate in diabetic rats are related to the various behavioural and psychological changes. The psychological changes observed in diabetes appear to persist even when the diabetic state is well-controlled with insulin administration ***(Michael et al., 1986)***. A decrease in the rate of 5-HT synthesis and changes in 5-HT neurotransmission have demonstrated to reduce 5-HT concentrations ***(Sandrini et al,. 1997)***

Anxiety is a neurological problem associated with diabetes mellitus. Muneoka and colleagues in 1997 revealed the correlation between diabetic anxiety and serotonergic systems. There is a decrease in the serotonergic response to stressful stimuli and the dysfunction of stress-elicited 5-HT release caused the increased expression of fear-related behaviour in diabetic rats ***(Miyata et al., 2007)***.

More recently, the concept of proinflammatory cytokine-induced hormone resistance has been thrust into the limelight because of the implication of this interaction in human disorders ranging from type 2 diabetes to clinical depression. The most studied example is TNF-α-induced insulin resistance, which was first described in the early 1990's. However, it is now known that proinflammatory cytokines also induce a state of resistance in other important hormone systems, including glucocorticoids ***(Avitsur R et al., 2005)*** GH ***(Lang CH et al., 2005)*** and IGF-I ***(Shen Wh et al., 2002).*** In the brain, they act to induce behavioral and motivational changes associated with sickness ***(Kelley KW et al., 2003)*** and depression ***(Schiepers OJ et al., 2005)***. A common thread in all of these examples is the proinflammatory cytokines.

Therefore, treatment of depression is necessary to improve the quality of life of diabetic patients to increase treatment compliance, and to decrease the risk of microvascular and macrovascular complications ***(Hofmann, 2010).*** Antidepressants have been reported to affect glucose homeostasis in diabetic individuals. Although diabetes risk is elevated for the major antidepressant classes, the risk posed by individual medications may vary widely ***(Jindal, 2009; Andersohn et al., 2009)***.

On the other hand, multiple evidences suggested that diabetes might attenuate the response to antidepressant classes ***(Jancin, 2003; Lustman et al., 2009; Hofmann, 2010).*** The benefits of tricyclic antidepressants for depression in patients

without other major medical problems are well-established, but the efficacy of any antidepressant agent for depression in diabetic patients remains unproven. Successful treatment of depression in diabetes may have positive effects on both mood and glucose regulation because of their observed relationship, but this possibility has not been studied systematically.

AIM OF THE WORK

The present study is designed to investigate the antidepressant effect of selective serotonin reuptake inhibitor (SSRI), 'fluoxetine' and tricyclic antidepressant (TCA), 'imipramine' in diabetic and non-diabetic rats exposed to chronic restraint stress. Additionally, hippocampal monoamines turnover, level of TNF-α and serum corticosterone will be measured. An attempt was made to detect any substantial difference in their impact on behavioral and neurochemical dysfunctions that might justify the preference of one antidepressant agent over the other for management of depression in type 2 diabetes.

REVIEW OF LITERATURE

Depression is a common mental disorder that presents with depressed mood, loss of interest or pleasure, feelings of guilt or low self-worth, disturbed sleep or appetite, low energy, and poor concentration. Depression has highly prevalent worldwide as according to WHO roughly 350 million people worldwide suffer from depression. This makes depression a major concern to the personal and economic welfare ***(WHO, 2012).***

Diabetes and Depression are major public health concerns leading to significant morbidity and mortality in the general population. According to the International Diabetes Federation there were 366 million people with diabetes in 2011, and its estimated to rise to 552 million by 2030 ***(Whiting DR et al, 2011).***

Depression is a common psychiatric disorder among diabetic Persons, the concurrence of depression and diabetes is a serious problem. There is growing evidence that depression is more common in people with type 2 diabetes whose risk of depression is 50–100% greater compared with control subjects without diabetes ***(Rubin et al., 2008);*** there is evidence of declined physical activity, higher obesity, and potentially more diabetes end-organ complications and impaired function ***(Caruso et al.,2000)***, mortality was found to be 54% higher in depressed

patients with diabetes in comparison to diabetic non-depressed patients ***(Egede et al., 2005)***. Besides, depression may impair control of glycemia and decrease adherence to oral hypoglycemic drugs ***(Ciechanowski et al., 2000)***, as well as higher rate of micro- and macro-angiopathic diabetic late complications and declined quality of life ***(Egede et al., 2005)***. On the other hand, the response to antidepressants is altered in diabetic patients **(*Lustman et al., 2009).*** Also treatment with antidepressants has been reported to disturb glucose homeostasis in diabetic individuals ***(Jindal, 2009)*.**

In fact patients with both diabetes and depression have a higher risk of death than patients with depression or diabetes alone. Behavioral and biological factors such as race, ethnicity and socioeconomic status are all identifiable risk factors for co-morbid depression in patients with diabetes mellitus. Depression not only often coexists with diabetes but also negatively effects treatment goals ***(Patel V. 2014)***.

Although there is evidence that supports the bidirectional association between depression and diabetes, the causal relationship is still unclear. Several studies that examined the role of depression in predicting the onset of type 2 diabetes mellitus, confirmed an increase in the risk of type 2 diabetes in patients with depression. However, this increase is not uniform and ranges between 32% and 60% ***(Golden SH et al, 2008)*** Similarly, several

studies that assessed the role of diabetes in increasing the risk of depression showed a relationship between the two with an incidence estimated between 15% to 24%, thus concluding that patients with type 2 diabetes are associated with a higher risk of depression ***(Nouwen A et al, 2010)***

- ***The bi-directional relationship between depression and Type 2 DM***

Type 2 DM leading to depression:

The increase in pro-inflammatory mediators associated with type 2 DM provides a probable link explaining the increased incidence of depression among patients with type 2 DM. It is important to note that type 2 DM is commonly accompanied with increased adiposity or obesity, a factor that has independently been associated with depression ***(Luppino et al., 2010).*** A diabetes or obesity related increase in inflammatory markers, hyperglycaemia and possibly hyperinsulinemia may contribute to a net pro-inflammatory state in many tissues. Access of pro-inflammatory mediators to the CNS may then lead to an activation of the pathways leading to the development of depressive symptoms. An animal study demonstrated in the db/db mouse model of diabetes that both the central and peripheral anti-inflammatory feedback responses to IL-1ß or LPS were reduced in diabetic mice ***(O'Connor et al., 2005).***